NOTES ON SOME SAPROPHYTIC SPECIES OF FUNGI, ASSOCIATED WITH DISEASED POTATO PLANTS AND TUBERS.

With Plates III and IV.

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During the progress of a series of investigations* on various diseases of the potato plant in Ireland, which have extended over several years, special attention was naturally devoted to the part played by parasitic fungi. In the course of the work, however, a number of saprophytic species associated with the parasites were met with, some of which were previously undescribed or imperfectly known; and a certain amount of attention was devoted to them.

It is proposed, in the following notes, to deal briefly with a few of these saprophytes; and the observations and descriptions which follow are based, to a large extent, on the characters and behaviour of the various species, when grown in pure culture, a single conidium or spore having been made the starting-point of the culture in each case. The study of micro-fungi in pure cultures offers many advantages, and had this method been available to and employed by earlier workers in mycology, many mistakes would have been avoided. It is clear, for reasons which need not be discussed here, that whenever possible this method should be adopted by workers in future.

I. NECTRIA INVENTA.

(Syn. *Verticillium cinnabarinum* Reinke et Berth.)

The more or less ubiquitous fungus *Acrostalagmus cinnabarinus* Corda† was, in 1879, re-named *Verticillium cinnabarinum* by Reinke and Berthold‡, who pointed out that Corda's generic name was founded upon an error of observation as to the manner in which conidia were produced. Nevertheless both Saccardo§ and Lindau|| retain Corda's original name.

* Reports on these investigations will be found in the Journal of the Department of Agriculture and Technical Instruction for Ireland, x.–xviii. 1910–1918.
† Icon. Fung. ii. p. 15, 1838.
‡ Zersetz. d. Kartoff. p. 63, 1879.
§ Syll. iv. p. 163, 1886.
|| Rabenh. Krypt. Fl. i. 8, p. 339, 1904.

This fungus is found quite frequently on the surfaces of decayed potato tubers in its well-known red conidial stage. It was grown in pure culture for a period of fifteen months on various nutrient media in the hope that some other form of fructification might develop in one or other of them. This, however; did not occur.

In July 1915 a number of old, diseased "seed" potatoes that had been thrown into a wet ditch in the spring were examined, more or less as a matter of curiosity. On the surfaces of several of these tubers a number of perithecia were found. A few of the latter were almost black; and microscopical examination showed that they probably belonged to some species of Gibberella. Most of the perithecia, however, were dark red in colour, somewhat similar to those of *Hypomyces Solani* R. et B. Since, however, a stroma was present it was evident that they belonged not to the genus Hypomyces, but to Nectria. Comparison with type material of *N. Solani* R. et B. showed that they were certainly not that species*.

Closely associated with the red perithecia was a copious development of the conidial stage of *V. cinnabarinum*, so close, in fact, that in some cases conidiophores of this fungus were present on the stroma, and even on the surfaces of the perithecia themselves. The question arose, therefore, as to whether this was a case of mere association, or whether the perithecia actually belonged to *V. cinnabarinum*. Many of the perithecia were still unripe, but in others ripe ascospores were present, and trials made in hanging-drops showed that the spores were viable.

Careful and continuous microscopic observations were made in five separate instances on the development of isolated individual ascospores in film cultures on the undersides of coverglasses, this being a more advantageous method of study than the hanging-drop. In each case the spore germinated and produced mycelium, which soon gave rise to conidiophores and conidia exactly similar to those of *V. cinnabarinum*, the growth from the original ascospore to the development of conidiophores and conidia being traced in unbroken sequence.

In another case the course of development from ascospore to conidia-production was followed under the microscope with a culture on a thin film of nutrient medium in a Petri dish. This is illustrated in Fig. 3, Plate III. The two-celled ascospore

* *N. Solani* does not appear to be a common species. I have never come across it and there is no reliable record of its occurrence in the British Isles. For years, however, in English phytopathological literature this fungus was credited, quite erroneously, with being the cause of the dry-rot of the potato tuber.

(*a*) is still discernible in the middle of the growth on the right, while on the left the young conidiophore borne on the hypha (*h, h*) is shown. Although this hypha is drawn in the figure with partially discontinuous walls, it was, as a matter of fact, continuous. The figure was drawn with the aid of a camera lucida which did not permit of the whole growth being outlined in one single field of view.

A stock pure culture was raised from a single ascospore, and this was made the basis of a series of sub-cultures on seven different media. A stock pure culture of *V. cinnabarinum* was raised from a single conidium of the fungus growing on a decayed potato, and sub-cultures from it were made on the same media. No differences, either macro- or micro-scopic, were discernible in the growths developed in the series of parallel cultures. Hence it is concluded that the perithecia, originally found on the surfaces of the rotting tubers in very intimate association with the conidial stage of *V. cinnabarinum*, are indeed the perfect state of fructification of this fungus*. This species, therefore, must be removed from the Fungi Imperfecti and be placed amongst the Ascomycetes.

The ripe perithecia are spherical or globular in shape and possess an ostiole, but are scarcely papillate. They are "cameo-brown"† in colour and bear short, stiff multi-cellular hairs or appendages on their upper halves. A stroma is present which in some cases bears only one perithecium, and in others more than one. The wall of the perithecium is several cells in thickness and more or less leathery, or cartilaginous in substance, not brittle. Long paraphyses are present in young perithecia, at any rate, but they are not easily made out and they disappear later on. They are more easily seen in the carefully teased out contents of a perithecium which is not too ripe than in sections. The asci are typically eight-spored but a lesser number sometimes occurs. The ascospores are arranged in one row and are 1-septate when ripe, single-celled when young. The walls of the paraphyses and asci appear to become mucilaginous, so that a fully ripe perithecium contains a mass of isolated ascospores embedded in a more or less gelatinous matrix. Fig. 4, Plate III, represents a longitudinal section (not quite a median one and slightly diagrammatic) through a perithecium and its stroma. The asci

* After this pure culture work was completed a case was met with in which one of the actual appendages of a perithecium had developed a conidiophore of *V. cinnabarinum* as a lateral branch, proceeding from near its distal end. This affords yet another link in the chain of proof that the fungus producing the perithecia is *V. cinnabarinum*.

† Ridgway, R. Color Standards and Color Nomenclature. Washington, D. C., 1912. Plate 28. 7″ k.

and the paraphyses are shown in more detail in Fig. 5, while Fig. 1 illustrates ripe and unripe ascospores and Fig. 2 shows ascospores germinating.

As regards nomenclature, the combination *Nectria cinnabarina* cannot, of course, be adopted, since this name is already in use for the well-known "Coral Spot" fungus. Since the perithecia were discovered more or less by accident the specific name *inventa* does not seem inappropriate. The characters of the fungus may be summed up, as follows:

NECTRIA INVENTA Pethybridge.

Peritheciis gregariis, globosis, atro-rufis, superiore parte pilosis, 300–500μ diam., paraphysibus filiformibus dein obsoletis, 3–4μ × 150μ; ascis cylindricis, vel cylindraceo-clavatis, 60–100μ × 4–6μ, octosporis; sporidiis monostichis, oblongis, hyalinis, 1-septatis, 4–5μ × 9–10μ.

Hab. In tuberibus putresc. *Solani tuberosi* in Hibernia. Status conid. sistit *Verticillii cinnabarini* R. et B. (*Acrostalagmi cinnabarini* Corda).

Inoculations with pure cultures, through wounds into living potato stems and tubers, did not result in any sort of infection; hence the fungus is a saprophyte, so far as the potato is concerned at any rate. Control inoculations into stalks of healthy growing potato plants were made at the same time with a pure culture of *Verticillium albo-atrum* R. et B. and hadromycosis was set up in each case.

II. COLLETOTRICHUM TABIFICUM.

(Syn. *Rhizoctonia tabifica* Hallier.)

In a paper published in 1875 Hallier* attributed the disease in potatoes known as "Curl" to the presence (chiefly in the pitted vessels of the wood) of a parasitic fungus which he named *Rhizoctonia tabifica*. Of course no cultures of the fungus were made, nor were any infection experiments carried out, and a critical study of the paper leaves no doubt in one's mind but that Hallier was dealing with at least two (if not more) distinct organisms. It is highly probable that the principal one of these was the fungus *Verticillium albo-atrum*, described later by Berthold† and Reinke, investigated more fully by the present author‡ still more recently, and shown to be the cause

* Hallier, E. Die Ursache der Kräuselkrankheit. Zeits. f. Parasitenkunde, iv. 1875, p. 97.

† Loc. cit., p. 67.

‡ Pethybridge, G. H. The Verticillium Disease of the Potato. Sci. Proc. Roy. Dublin Soc. xv. (N.S.), 7, 1916, p. 63.

of a specific disease, hadromycosis, which is one of that congeries of diseases included under the term "Curl" by older writers.

Hallier placed his fungus in the genus Rhizoctonia, because he supposed that it produced certain black, pseudo-parenchymatous bodies (provided with stiff black hairs or setae) which he found on the diseased plants and which he regarded as being sclerotia. There is, however, no proof that these bodies belonged to the fungus luxuriating in the wood vessels, and it is now certain that *V. albo-atrum* produces no such sclerotia.

Bodies corresponding to Hallier's sclerotia were very frequently met with on dead or dying portions of potato stalks, especially on the parts below ground, and often in plants attacked by *V. albo-atrum.* They have not been seen on tubers, although no special search for them there was instituted. They arise beneath the epidermis through which the setae first make their appearance, and one of them in this condition is illustrated in Fig. 6, Plate III. By bursting through, or by the decay of the superficial tissues of the stalk, the whole black body ultimately becomes exposed. The setae are just visible with the naked eye but are somewhat easily broken off on handling the material. Occasionally (when still present) the pith of potato stalks bearing these bodies is of an amethyst tinge.

In some respects these bodies do resemble sclerotia, and they may perhaps function as such temporarily. When young, at any rate, they appear to contain appreciable quantities of oil. But the walls of the hyphae making up the pseudo-parenchymatous tissue are not so thick as one commonly finds in sclerotia.

For some time I was in doubt as to what these bodies really were. Hence, portions of potato stalks bearing large numbers of them were placed in a moist dish, and kept under observation for a considerable time during the summer of 1915. After a while a moist, amethyst-coloured globule arose on the upper surfaces of a number of the black bodies resembling bacterial colonies, but made up, in reality, of masses of conidia. Sections through the bodies showed that they were solid, and that the conidia were produced from a compact surface layer of conidiophores. From this surface the setae also arise; their bases are surrounded by the conidiophores and become submerged in the uprising conidial mass, their tops alone protruding above it.

The structure, as thus revealed, is evidently the fructifying stage of a species of Colletotrichum, and the puzzle as to the nature of what Hallier figured as sclerotia of a Rhizoctonia may now be regarded as solved. A vertical section through a portion of one of these bodies is illustrated in Fig. 8, Plate III. The ripe spores are easily washed away but four detached ones are shown.

The once or twice septate conidiophores are arranged in a vertical palisade-like fashion and are absent when the "sclerotia" first emerge. They are usually simple, but a few branched ones have been observed. The setae are 1- to 3-septate.

The conidia are elongated, cylindrical, or slightly spindle-shaped, with somewhat bluntly pointed ends. Their size varies, but averages $21\mu \times 3\mu$. They are hyaline and contain either one central vacuole, or two situated towards the ends. They germinate by producing a germ tube from near one end; and after germination many of them, but by no means all, develop a transverse septum and become two-celled. After a few days' growth the mycelium produced gives rise to conidia borne on the ends of short branches and similar to those already described.

From a single conidium a pure culture was raised and sub-cultures were made on several different kinds of media. The mycelium produced is at first hyaline and almost wholly submerged. Old mycelium is smoke-coloured and bears appressoria (see Fig. 7, Plate III). A striking characteristic of all the cultures was the development of a beautiful amethystine fluorescence throughout the medium. After about a week's growth the development of the black bodies (always bearing setae) begins; and they are always produced in a series of concentric zones which are circular in Petri dishes, and oval on slants in test tubes. After a time many of these bodies produce on their surfaces the amethyst-coloured globule of conidia already described. The fungus thus produces conidia directly from the mycelium and also in fructifications of the Colletotrichum type. No other form of fructification appeared in the cultures. Its chief characters may be summarised as follows:

Colletotrichum tabificum (Hallier pro parte) Pethybridge.

Acervulis gregariis, primo sub-epidermicis, demum erumpentibus, atris, $100–270\mu$; conidiis continuis, cylindricis vel sub-fusiformibus, hyalinis, multitudine aggregata amethystinis apicibus abrupte aculeatis, $3\mu \times 21\mu$; basidiis fasciculatis, cylindricis, 1–2 septatis, $20–30\mu$ longis; setulis simplicibus, erectis, 1–3 septatis, atris $100–340\mu$ longis; appressoriis atro-fuligineis.

Hab. In stirpibus subterraneis mortuis, vel paene mortuis *Solani tuberosi*, in Hibernia.

Healthy living potato stalks and tubers were inoculated through wounds with a pure culture of the fungus, but beyond a strictly limited growth at the expense of the cells injured in making the wounds, no development occurred, and no trace of any kind of rot was set up. Since, however, the fungus was found occasionally on stalks not completely dead there may possibly be special conditions under which it behaves as a parasite or at least a feeble parasite.

After the above described study of *C. tabificum* had been made a paper was published by O'Gara* in which a new species of Colletotrichum (*C. solanicolum*) occurring on potato stalks was described. An attempt to obtain a culture of this species failed, but judging from the published description it is just possible that it may be identical with *C. tabificum.*

The setae in the latter appear to be longer and the conidia rather longer and narrower than in *C. solanicolum,* but too much stress must not be laid on these somewhat variable characters. Perhaps the most striking point of difference is that, although O'Gara grew his fungus in pure culture, he does not mention the development in the medium of any amethystine fluorescence such as is so characteristic of *C. tabificum,* and which he would scarcely have failed to observe had it been present. Nor, apparently, was the mass of spores borne by the acervuli of this colour. There appears to be some doubt as to whether *C. solanicolum* is parasitic or not, for no inoculation experiments are reported. But the fungus is stated to have been found on living as well as on dead potato stalks. No amethystine coloration of the pith of affected stalks was noted.

Taubenhaus† has also described a Colletotrichum, derived from potato tubers, which he regards as being identical with *C. solanicolum.* On priority grounds, however, he maintains that it should be called *C. atramentarium* since he regards this fungus as being equivalent to Frank's *Phellomyces sclerotiophorus* and this, in turn, to Berkeley and Broome's *Vermicularia atramentaria.*

Those of us who are familiar with the sterile *P. sclerotiophorus* and its fructifying stage *Spondylocladium atrovirens,* both as it occurs on the potato tuber in Europe and as it behaves in pure culture, will perhaps not readily concur in this view.

In his primary isolation experiment Taubenhaus obtained three fungi from the surface tissue of a potato tuber affected

* O'Gara, P. J. New Species of *Colletotrichum* and *Phoma.* Mycologia, vii. 1915, p. 38.

† Taubenhaus, J. J. A contribution to our knowledge of Silver Scurf (*Spondylocladium atrovirens* Harz) of the white potato. Mem. New York Bot. Gard. vi. 1916, p. 549.

with Silver Scurf, viz. a Colletotrichum, a Fusarium and *Spondylocladium atrovirens*. It seems possible that the sclerotia originally present may have been partly those of Spondylocladium (formerly known as Phellomyces) and partly those of a Colletotrichum. Or, the Colletotrichum may have been present in mycelial form (as also the Fusarium probably was) and was not killed by the preliminary treatment with mercuric chloride. The matter, at any rate, deserves further careful study before the view that Phellomyces is a Colletotrichum and not Spondylocladium can be accepted.

Vermicularia varians has been described by Ducomet* as producing a disease of potatoes in France, and the same disease apparently occurs in Australia† and South Africa‡. The published descriptions of this "dartrose" or "Black Dot" disease recall, to some extent, what one has seen of *Colletotrichum tabificum*; but whether there is any real connexion between these two fungi can only be decided by further study.

III. HYPOMYCES SOLANI Reinke et Berth.

This fungus was described by Reinke and Berthold§ in 1879, who stated that it was a pure saprophyte. Since the ascospores on germination gave rise to conidia which these authors took to be those of *Fusisporium Solani*, they regarded *Hypomyces Solani* as the perithecial stage of this species.

Fusisporium Solani Martius was renamed *Fusarium Solani* by Saccardo; and in the older literature this fungus was often regarded as a parasite and the cause of the "Dry Rot" of the potato tuber.

The investigations of recent years on the genus Fusarium have, however, shown that the name *F. Solani* has been used in the past for more than one species of this genus; and this doubtless explains some of the confusion that has arisen.

Hypomyces Solani in its perithecial stage does not appear to be very common. I have only found it on three or four occasions, and always on the surface of potato tubers in an advanced stage of decay; never on tubers still partially living. Several years ago I was able to obtain type material of the fungus which has been useful for purposes of comparison.

The object of the present study was to trace the complete life-cycle of *H. Solani* from ascospore to ascospore *in vitro*; to make a careful comparison between its conidial stage and some

* Ducomet, V. Ann. Ecole Nat. Agric. Rennes, ii. 1908.
† McAlpine, D. Potato Diseases in Australia, 1911, p. 92.
‡ Doidge, E. M. Agric. Journ. Un. South Africa, vii. No. 6, 1914, p. 879.
§ Loc. cit. p. 27.

of the species of Fusarium which commonly occur on the potato, and to settle the question as to whether it is a saprophyte or a parasite. The species of Fusarium with which it has been more closely compared in culture are *F. Solani* (Mart.) A. & W., *F. Martii* A. & W., *F. caeruleum* (Lib.) Sacc., *F. trichothecioides* Wr., *F. discolor* (A. & W.) var. *sulphureum* (Schlecht.) and *F. arthrosporioides* Sherb.

Large numbers of cultures on numerous different kinds of media have been studied and a variety of inoculation experiments carried out, but it is only proposed to give a brief account of some of the most important results here.

General growth in pure culture. The stock pure culture which served as the basis for all subsequent work was derived from a single ascospore. These spores germinate readily, each cell sending out a germ tube. A photograph of a germinated ascospore is shown in Fig. 1, Plate IV.

Growth on all media used was luxuriant, the aerial portion being usually copious and snow white. An eight-day old individual growing on wort-gelatine is illustrated in Fig. 2, Plate IV. No colour of any kind was ever developed, such as is characteristic of several species of Fusarium. The older growth, especially on media slanted in test tubes, is not fluffy or cottony, as a rule, but may rather be described as somewhat fibrous, that is to say, the hyphae combine laterally to form more or less pointed strands, roughly comparable with fibrous asbestos. None of the several species of Fusarium under study at the same time showed this kind of growth. The consorting hyphae are not merely mechanically adherent to one another but actual anastomoses or "H-shaped" unions are frequent. Cultures in Petri dishes show very distinct concentric zonation. One such culture is shown in Fig. 4, Plate IV. Each zone consists of a horizontal chiefly submerged vegetative growth of mycelium and a corresponding vertical, aerial growth, the latter consisting of conidiophores bearing conidia-globules and being formed during the night. Each zone requires twenty-four hours for its formation. Growth of this kind has not been observed in any species of Fusarium.

Conidiophores. These are usually long, erect, multicellular and simple. Conidiophores with one or two lateral branches are occasionally seen, but the mode and extent of branching does not resemble that typical of Fusarium. They are sometimes submerged in the medium. Very frequently they are aggregated together in the form of conical coremia, as described and figured by Reinke and Berthold; and much of the aerial growth of the fungus often consists of such coremia. The second type of much branched conidiophores figured by these authors* and obtained by them from pustules on tubers and stems evidently does not belong to Hypomyces, but to some species of Fusarium. Hypomyces does not appear to form pustules breaking through the skin of the potato like some species of Fusarium do, and attempts to produce them by pure culture inoculation of both healthy and sterilised tubers with intact skins failed.

Conidia. The conidia are produced singly at the apex of the conidiophore. When the conidium is ripe it becomes pushed on one side and does not immediately fall off. As the formation of conidia proceeds, a globule—at first elliptical, then spherical—forms at the tip of the conidiophore. This consists of a mass of conidia held together in a slightly alkaline fluid, just as occurs in some species of Verticillium. Not infrequently neighbouring globules of conidia in a culture touch one another and coalesce, forming thus a much larger globule which is then supported on several coremia. These globules are seen in Fig. 3, Plate IV, and they can just be discerned with the naked eye in Figs. 2 and 4, Plate IV.

After the first conidium is formed the protoplasmic contents of the conidiophore contract slightly, and a minute, slightly expanded collar remains at the extreme tip. The conidium next arising grows up and becomes seated in this collar, looking in its early stages like an egg in a cup. (See Fig. 10, Plate III.) When the conidium has reached its full size separation occurs at its base between its protoplasm and that of the conidiophore and a slight gap is seen. Subsequently the base of the conidium and the slightly contracted contents of the conidiophore each develop a thin wall. Somewhat the same kind of thing was described for *Fusarium caeruleum*† and accounts for the development of the foot-like base of the conidium often seen in Hypomyces as well as in several species of Fusarium.

Typical conidia of *Hypomyces Solani* are 3-septate, i.e. 4-celled, but forms with less or more septa are not uncommon. The average of many measurements of typical 3-septate forms was found to be $38\mu \times 6{\cdot}2\mu$. Similar measurements for *F. Solani* were found to be $30\mu \times 5{\cdot}3\mu$ so that the conidia of the latter are considerably smaller than those of *Hypomyces Solani*. Examples of them are shown in Fig. 9, Plate III. On germination the two terminal cells of the conidium invariably produce germ tubes first; the intermediate ones either later or not at all. In *F. Solani* the cells of the conidium were observed to germinate more or less simultaneously.

* Loc. cit. Pl. I, Figs. 5 and 6.

† Pethybridge and Lafferty. Sci. Proc. Roy. Dublin Soc. xv. (N.S.), No. 21, 1917, p. 204.

Chlamydospores. Reinke and Berthold refer to these as macroconidia and to the fusiform conidia as microconidia, following Tulasne's terminology. As a matter of fact the chlamydospores are smaller than the conidia, and there seems no adequate reason for retaining these somewhat obsolete terms. There is practically nothing to add to Reinke and Berthold's description of these spherical spores. They were produced in considerable numbers in all cultures but not so freely as the conidia. They developed extraordinarily abundantly in a sterilised cold water extract of ground Quaker Oats.

Their walls are thicker than those of the conidia and they are often more or less "warty," although this irregular thickening varies somewhat in different media and is sometimes completely absent. They germinate readily enough, but probably are more resistant to adverse conditions than the conidia and may serve, therefore, as resting spores. No experiments, however, were made on this point. A germinating chlamydospore is illustrated in Fig. 11, Plate III.

Development of Perithecia. During the first three or four months that the fungus was cultivated no perithecia were formed on any of the media used. Since they occur naturally on rotting potato tubers, special attention was devoted to culture on these. The fungus was planted on tubers affected with blight (*Phytophthora infestans*) both sterilised and unsterilised, as well as on sterilised tubers affected with "Pink Rot" (*P. erythroseptica*). Luxuriant growth developed in all cases but no perithecia were formed, although the cultures were kept under observation for fifteen months.

During the winter of 1913–14, the detailed culture work was suspended, but the stock culture was kept going by transfers at monthly intervals, mostly on oat extract agar, all the intermediate transfers being kept. After the fungus had been in culture for nearly a year perithecia began to develop both in some of the older transfers, which had been kept, and in the more recent sub-cultures on oat extract agar.

It would appear, therefore, that the fungus requires a more or less prolonged period of growth under artificial conditions, before it becomes stimulated to produce its perfect form of fructification. Having once reached this stage, the production of perithecia proceeds more rapidly. Thus, from a culture in which perithecia were present sub-cultures were made on sterilised potato stalks and sterilised portions of tubers, and within a month perithecia were developed. They were also formed on oat extract agar, Quaker Oat agar and beer-wort gelatine, but not quite so rapidly. In no case were they formed in these media as freely and abundantly as on decaying tubers under natural conditions.

The form and structure of the perithecia have been described in detail by Reinke and Berthold, and there is little to be added in this connexion. When ripe the ascospores are expressed through the mouth of the perithecium in the form of a yellow mass. Perithecia with two necks have occasionally been observed in naturally growing material but they were not seen in any of the cultures.

Inoculation Experiments. Healthy living potato stalks, tubers and rhizomes were inoculated at various times and repeatedly with ascospores, conidia and mycelium bearing both conidia and chlamydospores, but no infection occurred in any single case and no rot was set up. *Hypomyces Solani*, therefore, is a saprophyte which in addition to its perithecial stage produces conidia and chlamydospores resembling in some respects those produced by certain species of Fusarium. There are, however, pronounced differences between typical species of Fusarium and the conidial stage of Hypomyces, and it is concluded that *H. Solani* is not the perithecial stage of *Fusarium Solani* or of any other species of Fusarium.

IV. TWO NEW SPECIES OF VERTICILLIUM.

In a previous paper* dealing with the disease of the potato plant caused by *Verticillium albo-atrum* R. et B. attention was called to the discovery of two new species of Verticillium, occurring on the surface of potato tubers, which, in the absence of infection experiments or study in cultures, might easily be mistaken for *V. albo-atrum*. Indeed, it seems not at all unlikely that it was the presence of one or the other of these species on the surface of the tuber which led Berthold and Reinke into the error of supposing that *V. albo-atrum* did not actually enter the tuber, as it has now been proved to do, but reached the developing sprouts from without, after having traversed the outer surface of the tuber. A brief description of these two species seems, therefore, desirable.

One of them, which it is proposed to call *V. nubilum*, was found at a spot on the surface of a tuber attacked by *Phytophthora infestans* where the skin had received a slight mechanical injury; the other, *V. nigrescens*, on the skin of a tuber affected with ordinary "scab." Both were obtained in pure culture, starting from a single conidium in each case, and both were grown on a large number of different solid and liquid media

* Pethybridge, G. H. The Verticillium Disease of the Potato. Sci. Proc. Roy. Dublin Soc. xv. (N.S.), No. 7, 1916, p. 75.

and the growths compared with one another and with that of *V. albo-atrum* on the same media.

In their conidial form all three species are much alike, so far as their aerial parts are concerned. The conidiophores are very similar in each case. The size of the conidia in each species varies considerably, but parallel cultures on the same media showed that those of *V. albo-atrum* were on the average the smallest, and those of *V. nubilum* the largest; while those of *V. nigrescens* were intermediate. The measurements were made on what were considered to be the predominating type of conidia, omitting the extreme forms in each case.

The most striking differences are to be found in those portions of the growths which are submerged in the culture medium. In all three cases this submerged growth becomes dark after a time, and finally almost black. In the case of *V. albo-atrum* this darkening, as is well known, is caused by the turning black of the submerged hyphae. In *V. nubilum* and *V. nigrescens*, on the other hand, it is due, not to any change in the colour of the submerged mycelium, but to the production of large numbers of what may be regarded as chlamydospores which develop very dark, almost black, walls.

The chlamydospores of *V. nubilum* are more or less spherical cells hyaline at first, but soon developing thickish, dark brown or black walls. They may be borne singly or in groups of up to seven or so. They may be terminal but are frequently intercalary in rows of three or four or more. Examples are illustrated in Fig. 5, Plate IV. They are not, however, produced on all media and are usually absent in those containing gelatine. The mycelium on which they are formed remains visible for a long time.

The chlamydospores of *V. nigrescens* are considerably smaller than those of *V. nubilum*. They may be spherical, oval or somewhat pear-shaped. Generally they are single-celled, but occasionally they are septate. Many of them arise in an intercalary fashion by the thickening and blackening of non-contiguous cells in a hypha, and they retain, more or less, the shape of such cells. Often they are laterally sessile and then generally spherical. The cells of the hyphae which do not become chlamydospores become rather indistinct in old cultures, and the general impression is that of a row of irregular beads arranged at unequal intervals along, or at the side of, a faintly visible band. Examples are illustrated in Fig. 6, Plate IV. They are developed in very large numbers; and the blackening of the medium produced by them is considerably more intense than that caused by the two other species.

Although the two fungi were kept continuously in culture for three years, no reproductive organs, other than conidia and chlamydospores, were ever observed.

A considerable number of inoculation experiments were carried out with these two species on living potato stalks and tubers. In no case did infection occur and there was no indication of any ability to invade or grow in the vessels of the wood such as characterises the parasitic *V. albo-atrum.*

The characters of these two species may be summed up as follows:

VERTICILLIUM NUBILUM Pethybridge.

Mycelio albo effuso; ramis fertilibus ascendentibus, verticillatis; conidiis continuis, oblongatis, hyalinis, magnitudine varia plerumque $9\mu \times 3\mu$; hyphis in matrice submersibus chlamydosporis moniliformis vel conglobatis subglobosis, atris $8{\cdot}5$–12μ diam.

Hab. In tuberis putrescentibus *Solani tuberosi*, in Hibernia.

VERTICILLIUM NIGRESCENS Pethybridge.

Mycelio albo effuso; hyphis fertilibus erectis, ramis verticillatis; conidiis hyalinis, continuis, oblongatis, magnitudine varia plerumque $7\mu \times 2\mu$; mycelio in matrice submerso, chlamydosporis terminalibus, vel lateralibus, vel intercalariis, aut globosis, circ. $4{\cdot}3\mu$ diam., aut sub-ovalis, circ. $6\mu \times 4\mu$ atris.

Hab. In tuberis scabiosis *Solani tuberosi*, in Hibernia.

V. LANGLOISULA MACROSPORA A. L. SM.

When examining blighted potato foliage in the search for possible oospores of *P. infestans,* isolated brown spores were again and again met with, the origin and identity of which were not clear. The same spores have also been met with on blighted tubers.

No difficulty was experienced in getting a pure culture from these spores, and the resulting fungus was identified by Mr. J. Ramsbottom and Miss A. Lorrain Smith as *Langloisula macrospora* by comparison with type material in the British Museum. This species was described in 1901 by Miss Smith* who found it spreading over a grass seed in the germinating case.

On agar media the submerged mycelium is hyaline and about 3μ thick. Over the surface of the medium much larger hyphae about 9μ thick run, and from these conidiophores arise here and there in more or less isolated tufts or balls. The

* Smith, A Lorrain. Fungi found on farm seeds when tested for germination; with an account of two Fungi new to Britain. Journ. Roy. Microscop. Soc. 1901, p. 617. In the description in the text the species-name *heterospora* is given but this is probably a misprint for the name *macrospora* which is applied to the figures on the accompanying plate and which is given to the fungus in the account published in Trans. Brit. Myc. Soc. i. 1902, p. 194.

hyaline conidiophores develop in a peculiar sympodial fashion which give them a zig-zag appearance as illustrated in Fig. 12, Plate III.

The conidia are egg-shaped and brown in colour. The wall is slightly thickened but it is not nearly so thick as would appear from Miss Smith's drawings. Nor is it so strongly thickened as is shown in figures of the conidia of *L. spinosa* Ell. et Everh. In young conidia the wall is quite smooth and not warty. In old cultures the wall is slightly rough, but even in this condition can scarcely be described as warted. Careful examination of old conidia shows that the outermost portion of the wall is not continuous over the whole conidium and the slight roughness is due to this fact.

The conidia germinate easily, a germ tube being produced at the narrower end through a thin place in the wall at the point where the conidium was attached to the conidiophore. A germinating conidium is illustrated in Fig. 13, Plate III.

Attempts were made to infect living potato leaves (both intact and wounded) as well as stems and tubers, but without success. The fungus is a saprophyte.

As regards the systematic position of the fungus, it seems doubtful whether it should really be placed in the genus Langloisula. Its conidiophores are not dichotomously branched as in the type of that genus, and its conidia are not so thick walled. Miss Smith and Mr. Ramsbottom suggested to me that it would perhaps be better placed in the genus Monopodium of Delacroix*. Material was sent to Paris for comparison with Delacroix's specimens, but unfortunately no type material of them had been preserved.

M. Arnaud was good enough to look into the matter, however, and found that the specimen sent agreed exactly with an unpublished drawing by M. Griffon which the former believed to represent Delacroix's Monopodium. M. Arnaud further suggested that both the Monopodium of Delacroix and *Langloisula macrospora* were probably identical with *Acremoniella atra* Corda, and pointed out that the specimen of Langloisula forwarded resembled Saccardo's† drawing of this species rather more closely than the original one of Corda‡.

In concluding these notes I desire to acknowledge gratefully the assistance given me, in cultural and other work with the organisms described, by Mr. H. A. Lafferty, A.R.C.Sc.I., who was also good enough to prepare the drawings for the figures on Plate III.

* Bull. Soc. Myc. France, vi. 1890, p. 99.
† Fungi Italici. No. 713.
‡ Icon. Fung. i. Tab. III, fig. 168.

EXPLANATION OF PLATES.

Plate III.

(All figures were drawn with the aid of a Zeiss Camera Lucida.)

Fig.

1. Unripe (single-celled) and ripe (two-celled) ascospores of *Nectria inventa*. × 547.
2. Ascospores of *N. inventa* in various stages of germination in tap water. × 547.
3. An ascospore (*a*) of *N. inventa*, the germination and subsequent development of which on a cover-glass film was followed uninterruptedly under the microscope. The hypha *h, h* was continuous and developed on the left a characteristic conidiophore bearing conidia of *Verticillium cinnabarinum*. × 333.
4. Vertical section through a perithecium of *N. inventa* on its stroma. The section is not quite median and therefore does not pass through the ostiole, although the near presence of the latter is seen from the arrangement of the cells at the top. For the sake of clearness a large portion of the contents of the perithecium has been omitted. In an actual section the paraphyses are not nearly so clearly defined as is shown, owing to crowding. The mycelium permeating the cork-cells of the skin of the tuber on which the stroma sits, although present in the section, has been omitted from the drawing. × 55.
5. Portion of the contents of a perithecium of *N. inventa* teased out, showing the asci and septate paraphyses with rather swollen bases. × 340.
6. Young "sclerotium" (acervulus) of *Colletotrichum tabificum* on a potato stalk. The long black setae have penetrated through the epidermis but the remainder is still submerged in the tissue. × 91.
7. Appressoria of *C. tabificum* as developed in pure culture. × 560.
8. Vertical section through the acervulus of *C. tabificum*, as developed in pure culture. Four ripe conidia are shown isolated. The upper surface consists of a palisade-like layer of septate conidiophores, and the basal portions of two setae are shown. The lower part consists of rather thin walled pseudo-parenchymatous tissue. × 340.
9. Typical conidia of *Hypomyces Solani* from a 30-day old culture on oat extract agar. × 560.

Fig.

10. Two conidiophores of *H. Solani* from a 30-day old culture on a sterilised potato stalk. The one on the right shows the "collar" at its apex, that on the left is in process of developing a fresh conidium. × 340.
11. Germinating chlamydospore of *H. Solani*. × 560.
12. Conidiophore of *Langloisula macrospora*. × 340.
13. Germinating conidium of *L. macrospora*. × 340.

Plate IV.

1. Portion of the mycelium produced from a single ascospore (seen in the centre) of *Hypomyces Solani* in four days on a wort-gelatine cover-glass film. × 200.
2. An 8-day old individual of *H. Solani*, developed from a single ascospore on a wort-gelatine. The very numerous globules of conidia can just be seen with the naked eye as black dots. × 10.
3. Portion of the individual shown in Fig. 2, showing the conidiophores with their terminal globules of conidia.
4. A culture of *H. Solani* on oat extract agar, eleven days old. The first six days' growth occupies the centre and is more or less blurred by the presence of the large numbers of conidia-globules, many of which have fused together. The growth during the subsequent five days is clearly zoned. The lighter circles consist of aerial conidiophores, bearing conidia-globules; the darker, wider bands consist of more or less submerged mycelium. A dark and a light band, forming one zone, developed each 24 hours, the light circle of conidiophores being produced last and during the night. × $1\frac{1}{4}$.
5. Groups of chlamydospores of *Verticillium nubilum* from a pure culture on oat extract agar. × 360.
6. Chlamydospores of *V. nigrescens* from a pure culture on oat extract agar. The mycelium which bore these spores is now practically invisible. × 310.